PRODUCTION OF BIODIESEL FROM MICRALGAE USING ULTRASOUND

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ABSTRACT

Biodiesel is a clean-burning environmentally friendly fuel. Since the combustion of biodiesel releases carbon dioxide into the air that is taken out of the air by plants, it is called carbon neutral. In fact, it releases less greenhouse gas than diesel and it is biodegradable so it does not cause great harm to the environment if there is a spill. The ultrasound has been proved that low intensity of sonication can improve the fermentation process instead of high intensity sonication. The objective of the study is to investigate the production of lipid from microalgae by applying ultrasound, determine kinetic parameters and determine the rate of production of lipid. *Nannochloropsis sp.* will be used in this study and cultured in f/2 medium. The cell will be treated with ultrasound where the culture will be pump to ultrasound and recycled back to the medium. The impact of different power and regiment of ultrasound sonication will be investigated. Duty cycles of 10%, 20% and, 40% will be used. The outcome is ultrasound can enhance the production of lipid from microalgae *Nannochloropsis sp.* The optimum power and intensity is selected by observing the cell reduction. The optimum intensity for this project is 10.2 watt/cm^2. *Nannochloropsis sp.* gives a better result in 10% of duty cycle with the intensity of 10.2 watt/cm^2. The power and the intensity is determined after observe the absorbance after sonication at various power and amplitude. The 20% and 40% sonication did not give a good result because it seems to give a intense condition for the microalgae to grow.

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ABSTRAK

Biodiesel adalah bahan api pembakaran bersih yang mesra alam. Pembakaran biodiesel mengeluarkan karbon dioksida ke udara ini dibawa keluar dari udara oleh tumbuh-tumbuhan, ia dipanggil neutral karbon. Malah, ia mengeluarkan gas rumah hijau berbanding diesel dan ia terbiodegradasikan supaya ia tidak menyebabkan kemudaratan yang besar kepada alam sekitar jika terdapat tumpahan. Ultrasound telah dibuktikan bahawa intensiti rendah sonikasi boleh meningkatkan proses penapaian dan bukannya sonikasi intensiti yang tinggi. Objektif kajian ini adalah untuk menyiasat pengeluaran lipid dari mikroalga dengan menggunakan ultrasound, menentukan parameter kinetik dan menentukan kadar pengeluaran lipid. *Nannochloropsis sp*. akan digunakan dalam kajian ini dan dikulturkan dalam medium f / 2. Sel akan dirawat dengan ultrasound dimana sel yang dikulturkan akan dipam ke ultrasound yang disetkan ke dalam bekas dan dialirkan kembali ke kelalang kon dua liter. Kesan kuasa yang berbeza dan rejimen sonikasi ultrasound akan disiasat. Kitaran tugas sebanyak 10%, 20%, dan 40% akan digunakan. Keputusannya yabng jelas adalah ultrasound boleh meningkatkan pengeluaran lipid dari mikroalga *Nannochloropsis sp.* Kuasa optimum dan intensiti dipilih dengan memerhatikan pengurangan sel. Keamatan optimum untuk projek ini adalah 10.2 watt/cm^2. *Nannochloropsis sp.* memberikan hasil yang lebih baik dalam 10% kitar sonikasi dengan keamatan sebanyak 10.2 watt/cm^2. Kuasa dan keamatan ditentukan selepas melihat keserapan selepas sonication pada kuasa dan amplitud pelbagai. Kitar sonikasi 20% dan 40% tidak memberikan hasil yang baik kerana ia seolah-olah memberi satu keadaan sengit untuk mikroalga untuk berkembang.

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LIST OF ABBREVIATIONS

cm	Centimetre
CO_2	Carbon Dioxide
FAME	Fatty Acid Methyl Ester
hr	Hour
L	Litre
m	Metre
mg	Milligram
ml	Millilitre
min	Minute
N	Nitrogen
NaOH	Sodium hydroxide
Kg	Kilogram
P	Phosphorus
rpm	Revolutions per minute
s	Second
Si	Silicon
TAGs	Triglycerides
V	Volume
W	Power
%	Percentage
℃	Celsius Degree
μ	Specific growth rate

CHAPTER 1

INTRODUCTION

1.1 Background of Proposed Study

As energy demands increase and oil reserves begin to weaken in their stability, the need for a reliable renewable fuel source increase. There are many options such as bioethanol, biodiesel, and green diesels have the capability of providing a fuel source. Microalgae have become a source alternative energy in this modern world. Microalgae are microscopic algae, typically found in freshwater and marine systems.

It uses photosynthesis process to survive and important for life on earth. Microalgae can be used as a potential oil source due to its faster growth rates, high oil content and the ability to be harvested frequently over a long period of time (Haag and A.L, 2007). It produces storage lipids in the form of triacyglycerols (TAGs) (Balasubramanian et al, 2011). Biodiesel can be synthesized from triacyglycerols (TAGs) through transesterification reaction by using acid or base and methanol. Transesterification is the reaction of a fat or triacyglycerols (TAGs) with an alcohol to produce esters and glycerin.

Ultrasound is sound of frequency greater than 20 kHz. Wave frequency is the number of repetitions (or cycles) per second of a defined vibration state at a fixed location in space. Yusuf Chisti (2003) said that ultra-sonication is commonly related with damaging the cells but evidence is emerging for beneficial effects of controlled sonication on conversions catalyzed by live cells.

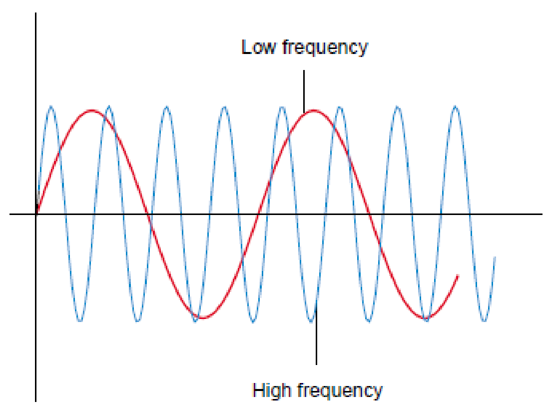

Figure 1.1 A Low And A High Frequency Wave

Source (Yusuf Chisti, 2003)

In this project, *Nannochloropsis sp* strain is selected for the experiment. After that, it involves the culturing of microalgae at a correct and optimum medium nutrient. Then, the cultured microalgae are set into two liters of conical flask and ultrasound is introduced. It is estimated the lipid production will increase. The amount of lipid is compared with and without ultrasound. Finally, the lipid obtained is compared with control growth to calculate and observe the changes. From Table 1.1, we can see that microalgae basically are an efficient source for biodiesel production.

Table 1.1 Comparison of microalgae with other biodiesel feed stocks

Plant source	Seed oil content (% oil by wt in biomass)	Oil yield (L oil/ha year)	Land use (m^2 year/kg biodiesel)	Biodiesel productivity (kg biodiesel/ha year)
Corn/Maize (*Zea mays* L.)	44	172	66	152
Hemp (*Cannabis sativa* L.)	33	363	31	321
Soybean (*Glycine max* L.)	18	636	18	562
Jatropha (*Jatropha curcas* L.)	28	741	15	656
Camelina (*Camelina sativa* L.)	42	915	12	809
Canola/Rapeseed (*Brassica napus* L.)	41	974	12	862
Sunflower (*Helianthus annuus* L.)	40	1070	11	946
Castor (*Ricinus communis*)	48	1307	9	1156
Palm oil (*Elaeis guineensis*)	36	5366	2	4747
Microalgae (low oil content)	30	58,700	0.2	51,927
Microalgae (medium oil content)	50	97,800	0.1	86,515
Microalgae (high oil content)	70	136,900	0.1	121,104

Source (Teresa M. Mata et al, 2010)

1.2 Problem Statement

It is known that in normal microalgae growth, the microalgae are capable of producing certain amount of lipid for biodiesel production. During the normal microalgae growth, for it to produce certain amount of lipid it is taking too much time. So, it is predicted that by introducing the ultrasound during the microalgae growth, we are able to get higher amount of lipid for biodiesel for the same amount of substrate. The specific growth rate of *Nannochloropsis sp*. is quite slow compared to other strain.

1.3 Research Objectives

The objectives of the production of biodiesel from algae using ultrasound by are:

1.3.1: To determine kinetic parameter of microalgae.

1.3.2: To identify and determine the rate of production of lipid by algae by using ultrasound.

1.4 Research Questions

The proposed study is constituted to find answers of the following questions:

1.4.1: How the production of lipid from algae changes when ultrasound is used?

1.4.2: What are suitable the power used for algae biomass production?

1.4.3: What are the suitable conditions of ultrasound to enhance lipid production?

1.5 Scope of Proposed Study

In order to achieve the objectives of the study, the scope of the proposed study are to culture the *Nannochloropsis sp* .in f/2 medium to grow at optimal condition. Furthermore, the lipid production by ultrasound is done by manipulating the sonication regiment. The sonication regiment can be manipulated by changing the duty cycle and the intensity. The intensity is calculated by using, $I = P/A$. The duty cycle and power of ultrasound is manipulated in order to enhance lipid production.

1.6 Expected Outcome

The ultrasound can increase the rate of production of lipid from microalgae. So that, the lipid obtained can be changed into a better renewable energy; biodiesel.

1.7 Significance of Proposed Study

The significance of the producing biodiesel from microalgae using ultrasound is to increase the biodiesel / lipid production and to improve the fermentation process of microalgae.

1.8 Conclusion

Lipid is extracted from the microalgae and the production of the lipid is increased by manipulating the suitable power and duty cycle of sonication. Since biodiesel is extracted from algae, it is a clean energy, renewable, non toxic and sustainable alternative to petroleum based fuels, and it is able to reduce toxic emissions when is burned in a diesel engine. Hence biodiesel can be a better replacement for fossil fuel.

CHAPTER 2

LITERATURE REVIEW

2.1 Microalgae

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their simple multicellular structure as shown in the Figure 2.1. Microalgae are present in all existing earth ecosystems, not just aquatic but also terrestrial. It represents a big diversity of species living in a wide range of environmental conditions. Microalgae reproduction occurs mainly by vegetative cell division, although sexual reproduction can occur in many species under appropriate growth conditions. Microalgae are the most primitive form of plants but the mechanism of photosynthesis in microalgae is similar to that of higher plants (Scott et al. , 2010)

The Figure 2.1 shows the basic overview of photosynthetic pathway of a microalgae. Precursor fatty acids are synthesized de novo in the chloroplast, using either carbon fixed during photosynthesis, or from an exogenous supply of organic carbon; the exact nature of what enters the chloroplast is unknown in algae (dashed line). Free fatty acids are exported from the chloroplast and then converted to TAGs in the endoplasmic reticulum (ER), where they bud off into oil bodies in the cytosol.

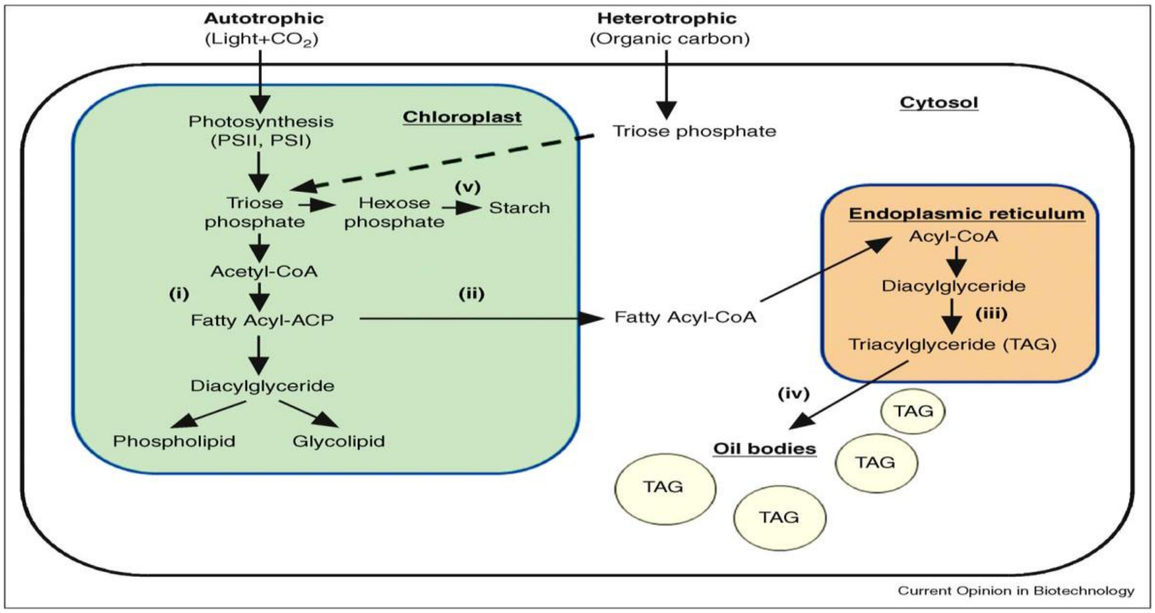

Figure 2.1 Basic overview of the pathway of carbon capture and lipid biosynthesis. Key: (i) = acetyl-CoA carboxylase (ACCase) and fatty acid synthase (FAS); (ii) = fatty acid thioesterases and acyl-CoA synthetases; (iii) = TAG biosynthesis enzymes, including acyl-CoA:diacylglycerol acyltransferase (DGAT); (iv) = oil body formation; and (v) = ADP-glucose pyrophosphorylase and starch synthase.

Source (Scott et al., 2010)

Microalgae consist of four major groups which are diatoms, green algae, blue green algae, and golden brown algae. Diatoms are among the most common and widely distributed groups of algae in existence. This group tends to dominate the phytoplankton of the oceans, but is commonly found in fresh and brackish water habitats. The cells are golden-brown because of the presence of high levels of fucoxanthin, which is a photosynthetic accessory pigment. Meanwhile in green algae, it is often referred to as chlorophytes. These are also quite abundant, especially in freshwater. They can occur as single cells or as colonies. In blue green algae, they have an organization and structure which is similar with bacteria. The function of these algae is fixation on nitrogen from atmosphere. Finally is golden brown alga which is similar to diatoms which respects to pigments and biochemical composition. Their usual reproduction is asexual by cell division and some species of this group have flagellation morphology.

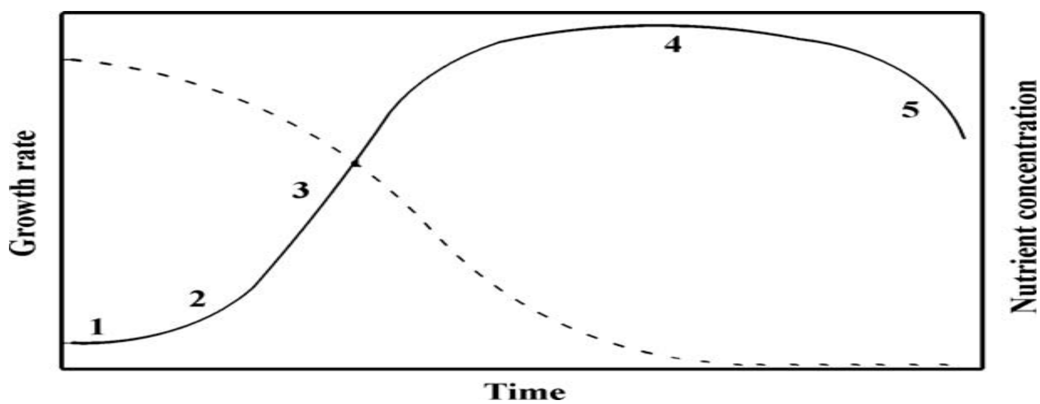

Figure 2.2 Schematic representation of algae growth rate in batch culture (solid line) and nutrients concentration (dashed line) 1. Lag phase,2. Exponential growth phase, representing the maximum growth rate, 3. Linear growth phase, 4. Stationary growth phase, 5. Decline or death phase.

Source (Mata et al., 2010).

According to Debirmas (2011), microalgae can be the best option for production of microalgae because:

1. Algae are the fastest growing plants in the world. Microalgae have much faster growth rates than other crops.
2. The cost of harvesting of microalgae is cheaper than other crops.

3. Microalgae are capable of fixing CO_2 in the atmosphere, thus help the reduction of increasing atmospheric CO_2 levels which are now considered a global problem.
4. Microalgae are easily biodegradable and they can be chemically treated easily.
5. Algae cultivation is not complex where they can grow practically in every place where there is enough sunshine.

2.1.1 Selection of Microalgae for Biodiesel

The Table 2.1 shows the lipid productivity of different strain. The strain is chosen according to their availability Some strain seems not to be available in the selected area. Hence, that is why raw material availability is taken into consideration.

Table 2.1 Lipid content and productivities of different microalgae species

Marine and freshwater microalgae species	Lipid content (% dry weight biomass)	Lipid productivity (mg/L/day)	Volumetric productivity of biomass (g/L/day)	Areal productivity of biomass ($g/m^2/day$)
Ankistrodesmus sp.	24.0–31.0	–	–	11.5–17.4
Botryococcus braunii	25.0–75.0	–	0.02	3.0
Chaetoceros muelleri	33.6	21.8	0.07	–
Chaetoceros calcitrans	14.6–16.4/39.8	17.6	0.04	–
Chlorella emersonii	25.0–63.0	10.3–50.0	0.036–0.041	0.91–0.97
Chlorella protothecoides	14.6–57.8	1214	2.00–7.70	–
Chlorella sorokiniana	19.0–22.0	44.7	0.23–1.47	–
Chlorella vulgaris	5.0–58.0	11.2–40.0	0.02–0.20	0.57–0.95
Chlorella sp.	10.0–48.0	42.1	0.02–2.5	1.61–16.47/25
Chlorella pyrenoidosa	2.0	–	2.90–3.64	72.5/130
Chlorella	18.0–57.0	18.7	–	3.50–13.90
Chlorococcum sp.	19.3	53.7	0.28	–
Crypthecodinium cohnii	20.0–51.1	–	10	–
Dunaliella salina	6.0–25.0	116.0	0.22–0.34	1.6–3.5/20–38
Dunaliella primolecta	23.1	–	0.09	14
Dunaliella tertiolecta	16.7–71.0	–	0.12	–
Dunaliella sp.	17.5–67.0	33.5	–	–
Ellipsoidion sp.	27.4	47.3	0.17	–
Euglena gracilis	14.0–20.0	–	7.70	–
Haematococcus pluvialis	25.0	–	0.05–0.06	10.2–36.4
Isochrysis galbana	7.0–40.0	–	0.32–1.60	–
Isochrysis sp.	7.1–33	37.8	0.08–0.17	–
Monodus subterraneus	16.0	30.4	0.19	–
Monallanthus salina	20.0–22.0	–	0.08	12
Nannochloris sp.	20.0–56.0	60.9–76.5	0.17–0.51	–
Nannochloropsis oculata.	22.7–29.7	84.0–142.0	0.37–0.48	–
Nannochloropsis sp.	12.0–53.0	37.6–90.0	0.17–1.43	1.9–5.3
Neochloris oleoabundans	29.0–65.0	90.0–134.0	–	–
Nitzschia sp.	16.0–47.0			8.8–21.6
Oocystis pusilla	10.5	–	–	40.6–45.8
Pavlova salina	30.9	49.4	0.16	–
Pavlova lutheri	35.5	40.2	0.14	–
Phaeodactylum tricornutum	18.0–57.0	44.8	0.003–1.9	2.4–21
Porphyridium cruentum	9.0–18.8/60.7	34.8	0.36–1.50	25
Scenedesmus obliquus	11.0–55.0	–	0.004–0.74	–
Scenedesmus quadricauda	1.9–18.4	35.1	0.19	–
Scenedesmus sp.	19.6–21.1	40.8–53.9	0.03–0.26	2.43–13.52
Skeletonema sp.	13.3–31.8	27.3	0.09	–
Skeletonema costatum	13.5–51.3	17.4	0.08	–
Spirulina platensis	4.0–16.6	–	0.06–4.3	1.5–14.5/24–51
Spirulina maxima	4.0–9.0	–	0.21–0.25	25
Thalassiosira pseudonana	20.6	17.4	0.08	–
Tetraselmis suecica	8.5–23.0	27.0–36.4	0.12–0.32	19
Tetraselmis sp.	12.6–14.7	43.4	0.30	–

Source (Mata et al, 2010)

From the Table 2.1, we can see that *Botryococcus braunii* contains highest lipid content however its lipid productivity is very low. The aim of the study is to find the strain that have faster growth rate. Furthermore, *Botryococcus braunii* has been found unsuitable for biodiesel production, because its hydrocarbons have a chain length greater than C30, while vegetable oils currently used for biodiesel are mainly C16 and C18. (Griffiths et al. 2009). The suitable microalgae would be *Nannochloropsis*. This is because, a research shows that the characteristics of fatty acid from *Nannochloropsis* is suitable for biodiesel and it has low viscosity (Miri Koberg,2011) . In addition, it also has high lipid productivity. Furthermore, *Nannochloropsis* can be obtained from University Malaysia Pahang. So, this shows that *Nannochloropsis* will be a suitable for this project.

According to Chojnacka et al.,(2004) microalgae can be culture through:

1. Photo autotrophically, using light as an only energy source that is converted to chemical energy through photosynthetic reactions.
2. Heterotrophically, utilizing only organic compounds as carbon and energy source.
3. Mixotrophically, performing photosynthesis as the main energy source, though both organic compounds and CO_2 are essential.
4. Photoheterotrophycally, also known as photo assimilation, describes the metabolism in which light is required to use organic compounds as carbon source. The photo heterotrophic and mixotrophic metabolisms are not well distinguished, in particular they can be defined according to a difference of the energy source required to perform growth and specific metabolite production.

Liam Brennan and Philip Owende (2011) justified that photoautotrophic production is the only method which is technically and economically feasible for large-scale production of algae biomass.

2.1.2 Water selection

To have a constant medium so that there will be no change in the medium nutrient, artificial sea water will be used. The artificial sea salt concentration is 17.5 g/l (Illman et al., 2000)

2.1.3 Selection of Medium

There are many medium for microalgae culture such as DYIY medium, f/2 medium, Bold Basal medium, D medium, and BG – 11 medium. Among the major considerations to grow microalgae are pH, concentration of major nutrients, nitrogen source, possible organic or growth factors for enrichment and micro nutrient composition (Shilpakar et al, 2010). *Nannochloropsis* will grow better in f/2 medium because of the optimum nutrients for its metabolism (Chiu et al., 2009). This shows that the f/2 medium should be the preferred choice for the laboratory culture (Phukan et al.,2011).

2.2 Microalgae for Biodiesel

Biodiesel refers to a vegetable oil or animal fat-based diesel fuel consisting of long-chain alkyl (methyl, propyl or ethyl) esters. Biodiesel is typically made by chemically reacting lipids vegetable oil or animal fat with an alcohol producing fatty acid esters. In this project we considering lipid from microalgae. Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops (Demirbas and Fatih Demirbas 2011). The lipid from microalgae is known as triacyglycerols which is also known as TAG. This triacyglycerols is reacted with alcohol to produce esters and glycerol. This process is known as transesterification. Debirmas MF (2010) stated that the biodiesel transesterification reaction is :

$$\text{Triglycride} + 3\ \text{methanol} \underset{}{\overset{\text{Catalyst}}{\rightleftharpoons}} \text{glycerine} + 3\ \text{methyl esters} \tag{1.1}$$

Demirbas and Fatih Demirbas (2011) justified that an excess of methanol is used to force the reaction to favor the right side of the equation 1. The excess methanol is later recovered and reused. The algae that are used in biodiesel production are usually aquatic unicellular green algae because this type of algae is characterized by high growth rates, high population densities can double its biomass in less than 24 hour and have huge lipid contents (Demirbas and Fatih Demirbas 2011). Hence, this high yield, high density biomass is ideal for intensive agriculture and may be an excellent source for biodiesel production.

Mustafa Balat (2011) has done a research in biodiesel and has said that non-edible plant oils have been found to be promising crude oils for the production of biodiesel. In addition to that, Mustafa Balat (2011) also said that microalgae have long been recognized as potentially good sources for biofuel production because of their high oil content and rapid biomass production.

Biodiesel can be used alone, or blended with petro diesel. Biodiesel can also be used as a low carbon alternative to heating oil. Blends of biodiesel and conventional hydrocarbon-based diesel are products most commonly distributed for use in the retail diesel fuel marketplace. B100 is referred as 100% biodiesel meanwhile B20 referred to 20% biodiesel and 80% of petrol diesel. According to biodiesel standard published by the American Society for Testing Materials (ASTM), biodiesel from microalgae oil is similar in properties to the standard biodiesel, and is also more stable according to their flash point values (Demirbas and Fatih Demirbas 2011). Biodiesel produced from algae is a new sustainable energy source substituted for petroleum diesel. Producing biodiesel from algae has been touted as the most efficient way to make biodiesel fuel.

2.3 Ultrasound

Ultrasound, or sound of frequency greater than 20 kHz, is inaudible to the human ear. Although this limit varies from person to person, it is approximately 20 kilohertz (20,000 hertz) in healthy, young adults. The production of ultrasound is used in many different fields, typically to penetrate a medium and measure the reflection signature or supply focused energy. Similar to ultrasonic cleaning, biological cells including bacteria can be disintegrated. This has uses in biological science for analytical or chemical purposes and in killing bacteria in sewage. High power ultrasound can disintegrate corn slurry and enhance liquefaction and saccharification for higher ethanol yield in dry corn milling plants. This is because high power ultrasounds produce cavitations that facilitate particle disintegration or reactions. Cavitation is a two-step phenomenon. The first step is the formation of vapor bubbles, and the second step is the collapse or implosion of the vapor bubbles. According to Zhaofeng Liang and his co-researches (2006) the first stage of cavitation is called stable cavitation and usually does not cause damage meanwhile full cavitation will sound like rocks flowing through the piping system and results in surface damage and called transient cavitation. According to Yusuf Chisti (2003) ultrasonication is generally associated with damage to cells but evidence is emerging for beneficial effects of controlled sonication on conversions catalyzed by live cells. Furthermore, Yusuf Chisti (2003) also stated that ultrasound has the potential for enhancing mass transfer within a cell. Recent studies have shown the low frequency ultrasonic irradiation can enhance penetrability of cell membrane and accelerate substance exchange because the cell may be hurt in some ways and make cell membrane flaw (Chuanyun et al., 2003). Ultrasonics can also be delivered via an ultrasonic horn, which is a popular method not for cleaning but for cell disruption, emulsification,and homogenizing of biological matter, where cavitation drives the actions (Parag Kanthale et al., 2008). Figure 2.3 shows different types of ultrasound horn for sonication process.